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# Trinucleotide Repeat Expansion in the FRAXE Locus Is Not Common Among Institutionalized Individuals With Non-Specific Developmental Disabilities

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**Expansion of a polymorphic GCC-repeat at the FRAXE locus has been associated with expression of chromosome fragility at this site and cognitive impairment in some individuals previously testing negative for CGG-repeat expansion in the fragile X mental retardation-1 (FMR1) gene. To determine the frequency of FRAXE triplet repeat expansion among persons with developmental disability, 396 individuals from two institutions were studied, all of whom were negative for FMR1 repeat expansion. Clinically, there was a wide range of mental impairment, with the majority (61.1%) being severely to profoundly affected. The distribution of FRAXE GCC-repeat numbers in the study population was 5–38: 28 (5.6%) with 10–14 repeats; 366 (73.8%) with 15–19 repeats; 74 (14.9%) with 20–24 repeats; 20 (4.0%) with 25–29 repeats; and 5 (1.0%) with 30–38 repeats, with no individuals demonstrating repeat expansion. One profoundly retarded male was found to have a deletion of about 40 bp. Southern blots of HindIII-digested DNAs from individuals with ≥26 repeats all showed normal patterns. These results suggest that FRAXE GCC-repeat expansion is not a common cause of developmental disability in institutionalized persons with mild to profound mental retardation.**



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## INTRODUCTION

Since the discovery of CGG-repeat expansion in the FMR1 gene as causal in the fragile X syndrome, some families expressing chromosome fragility in Xq27→Xq28 have been identified whose FMR1 CGG-repeat numbers were in the normal range. Most of these have subsequently been found to have trinucleotide-repeat expansion at one of two other folate-sensitive fragile sites, termed FRAXE [Knight et al., 1993, 1994; Hamel et al., 1994; Mulley et al., 1995] and FRAXF [Parrish et al., 1994; Ritchie et al., 1994]. FRAXE syndrome patients thus far reported differ from individuals with the classical fragile X syndrome (who express fragility at the FRAXA site) in several ways: there is no phenotypic overlap in terms of facial features, cognitive impairment is generally mild compared to the fragile X syndrome, and both males and females can be similarly affected. The presence of fragility and GCC-repeat expansion at the FRAXE site in affected, but not unaffected, members of the "FRAXE" families has led to the suggestion that hypermethylation of a CpG island adjacent to the FRAXE GCC-repeat region inactivates a nearby gene resulting in developmental disability, a mechanism analogous to that demonstrated for the FMR1 gene [Mulley et al., 1995]. In contrast, FRAXF expression and GCC-repeat expansion were found to be present in both affected and unaffected males from the same family [Parrish et al., 1994]. Thus, triplet repeat expansion at FRAXF does not appear to be associated with developmental disability.

The frequency of the FRAXE syndrome is not known since most persons chromosomally screened for fra-

gility in Xq27→q28 during the last 8–10 years were preselected based on subtle clinical anomalies seen in persons with the classical fragile X syndrome or because of a positive family history of mental retardation. To determine whether expansion of the FRAXE GCC-repeat is common among persons with developmental delay, we tested individuals from two institutions for the developmentally disabled who had previously been found to be negative for CGG-repeat expansion in the FMR1 locus [Holden et al., 1995]. None of the 396 males and females tested were found to be positive for FRAXE GCC-repeat expansion.

## METHODS AND MATERIALS

### Subjects

The subjects were residents at two regional centres for the developmentally delayed, previously described in Holden et al. [1995]. Known fragile X individuals were not included in this study.

### FRAXE Testing

Initial studies on the FRAXE GCC-repeat were done by PCR using the primers described [Knight et al., 1993], with the exception that blood spots [Holden et al., submitted] were used as the source of DNA for some of the initial PCR studies and the number of PCR cycles was then increased to 40. Amplification was carried out using 3 $\mu$l of template (from blood spots) or 200 ng of DNA in a 10 $\mu$l reaction containing 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 200 $\mu$M dATP, 200 $\mu$M dTTP, 200 $\mu$M dCTP, 50 $\mu$M dGTP, 150 $\mu$M 7-deaza-dGTP, 1.5 $\mu$Ci (alpha-$^{32}$)dCTP, 10% DMSO, 0.5 U Taq polymerase (BRL), and 5 pmoles of each of the primers. Primers were boiled for 5 minutes before adding to hot cocktail. Each reaction was overlaid with mineral oil and denatured at 95°C for 5 minutes followed by 30 (for DNA) or 40 (for blood spots) cycles of 95°C, 1.5 minutes; 67°C, 1 minute; and 72°C, 2 minutes. The final extension was at 72°C for 7 minutes. Aliquots of each reaction were mixed with formamide loading buffer and heated for 5 minutes at 95°C, and the products were separated by electrophoresis through a 4% denaturing polyacrylamide gel. Alleles were visualized by exposure to Kodak XAR-5 film.

Southern blotting of HindIII-digested DNA and probing with OxE20 [Knight et al., 1994] was done in all cases where there was no signal after PCR or the repeat size was ≥26 GCC's. Further, all female samples showing a single allele were examined by Southern blotting.

## RESULTS

### Subjects

Ninety-nine females and 298 males were tested for expansion of the GCC-repeat at the FRAXE locus. There was a wide range of clinical phenotypes, with 284 being severely or profoundly impaired and 46 having borderline to moderate mental retardation (Table I). The majority of individuals with a chromosome abnormality had trisomy 21.

TABLE I. Clinical Information on Institutionalized Individuals Tested for FRAXE GCC-Repeat Amplification

| Diagnosis | N |
|---|---|
| Borderline MR | 1 |
| Mild MR | 15 |
| Moderate MR | 30 |
| Severe MR | 69 |
| Profound MR | 215 |
| Unspecified level MR | 26 |
| PKU | 2 |
| Chromosomal abnormalities | 33 |
| Autism | 2 |
| Other known causes | 3 |
| Total | 396 |

### The Frequency and Distribution of FRAXE GCC-Repeat Numbers

The frequency and distribution of GCC-repeat numbers in the subjects, as determined by PCR across the repeat, are shown in Table II. The range of repeat sizes was from 5–38, with no individuals having amplification of the GCC-repeat. The most frequent allele had 15 GCC-repeats (35.2%). The next most common alleles were 16 (15.5%) and 18 (11.9%) repeats. All 21 individuals with an allele above 25 repeats were found to have a normal pattern on Southern blots (data not shown).

A single male had a relatively small PCR fragment size (Fig. 1A). Southern blotting confirmed this finding (Fig. 1B), indicating that he has a deletion of about 40 bp within the PCR-amplified region. He is profoundly mentally retarded and has a twin. No other family information is available at this time.

## DISCUSSION

No individuals with GCC-repeat expansion at FRAXE were identified in this study, a finding similar to that of Allingham-Hawkins and Ray [1995], who examined a consecutive series of 300 developmentally disabled males referred for FMR1 gene testing and found

TABLE II. The Distribution and Frequency of FRAXE GCC-Repeat Numbers Among Developmentally Delayed Subjects

| Repeat number | N | Repeat number | N |
|---|---|---|---|
| 1 | (1) | 21 | 17 |
| 5 | 1 | 22 | 8 |
| 8 | 1 | 23 | 12 |
| 10 | 1 | 24 | 13 |
| 11 | 4 | 25 | 4 |
| 12 | 4 | 26 | 5 |
| 13 | 5 | 27 | 6 |
| 14 | 14 | 28 | 2 |
| 15 | 175 | 29 | 3 |
| 16 | 67 | 30 | 1 |
| 17 | 34 | 31 | 1 |
| 18 | 59 | 35 | 2 |
| 19 | 31 | 38 | 1 |
| 20 | 24 | | |

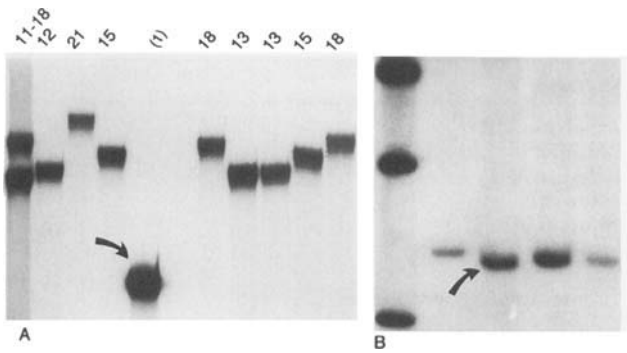


Fig. 1. **A:** PCR amplification of the FRAXE GCC-repeat region in ten mentally impaired individuals. Numbers at the top of each track indicate the number of GCC-repeats. Arrow points to the sample showing a deletion in the PCR product from one profoundly mentally handicapped male. The deleted allele is indicated by (1). **B:** Southern blot analysis of genomic DNA digested with HindIII and probed with OxE20. Arrow points to the approximately 40 bp deletion in the male identified in 1A.

to be negative at that locus. Whereas the present study included a large proportion of severely and profoundly mentally handicapped males and females, that by Allingham-Hawkins and Ray involved less severely affected males, almost 20% of whom had a learning disability or attention deficit disorder. Together, these studies examined a wide range of developmental disabilities and, if GCC-repeat expansion was a common cause of significant cognitive impairment, such individuals should have been identified. Given that the clinical phenotypes of individuals reported to have FRAXE GCC-repeat expansion [Dennis et al., 1992; Flynn et al., 1993; Knight et al., 1994] or deletion [Gedeon et al., 1995] are generally mild, further studies on large numbers of individuals with learning disabilities may lead to the identification of additional FRAXE families.

Knight et al. [1993] reported distributions of 6–25 repeats at FRAXE in normal and developmentally disabled individuals. The largest number of repeats in the Allingham-Hawkins and Ray [1995] study was 35. Our results extend this range from 5–38 repeats, with most individuals having 15 (35.2%), 16 (15.5%), or 18 (11.9%) repeats. We tested all individuals with repeat numbers ≥26 repeats using Southern blotting, and found that alleles in the 26–38 repeat range appear to be somatically stable.

Despite considerable effort in characterizing the FMR1 gene and its RNA and protein products, no role has yet been identified for the triplet repeat region in the 5′UTR of the FMR1 gene. Curiously, no individuals have been identified who lack only the repeat [based on findings of both special populations and our own general population screen of 2,072 alleles in males (Holden et al., in preparation)]. This observation, coupled with the fact that the triplet repeat region is conserved during evolution [Deelen et al., 1994], supports the notion that the region does have an important function. We have identified one profoundly mentally handicapped male who has a small (40 bp) deletion in the FRAXE region amplified during the PCR reaction. Given the observation that the majority of human genes containing CGG-repeats have them in their 5′UTRs [Riggins et al., 1992; Richards and Sutherland, 1992], with the CGG strand in the transcript, it is likely that the FRAXE GCC-repeat lies within the 5′UTR of a FRAXE gene. A candidate gene for FRAXE has recently been identified [Gedeon et al., 1995], but proof that this gene is related to the FRAXE CpG island identified by Knight et al. [1993, 1994] and Mulley et al. [1995] awaits isolation of the cDNA associated with the latter. If our subject lacks only GCC-repeats and does not produce a FRAXE-related mRNA or protein, it would suggest that 5′UTR-containing CGG-repeats have an important role in gene expression. Sequencing of the GCC-repeat region and family studies are underway to clarify this.

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